Chromatophore Responses in Relation to the Photoperiod and Background Color in the Hawaiian Ghost Crab, *Ocypode ceratophthalma* (Pallas)[1]

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THE FUNCTIONAL ACTIVITIES of chromatophores are classified as morphological when there is a change in the amount of pigment present over a period of time, and as physiological when there is a relatively rapid change due to changes in the degree of dispersion of the pigment. A primary chromatophore response is a response to a nonvisual stimulus, while a secondary response is a response to a visual stimulus (Fingerman, 1963:8).

The most common method of describing the degree of dispersion of the chromatophore pigments is that of Hogben and Slome (1931:12, fig. 1) using a one to five scale, where one corresponds to maximum concentration and five to maximum dispersion of the pigments, and the intermediate stages are described as two, three, and four.

The present study deals with the ability of the Hawaiian ghost crab, *Ocypode ceratophthalma* (Pallas), to maintain a rhythmic physiological chromatophore response with different periods of light and darkness and on different backgrounds.

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MATERIALS AND METHODS

Male and female ghost crabs were collected from three different areas on the island of Oahu, where the beaches are composed of very fine sand presenting a white background. Large crabs, with a carapace length of more than 22 mm, and medium crabs, with a carapace length of less than 22 mm, were collected. A length of 22 mm was chosen as the dividing point between large and medium crabs because ghost crabs begin to develop eye stiles, a characteristic of mature crabs, when they reach a carapace length of more than about 20 mm (Crane, 1941:303). Several attempts were made to use small crabs with a carapace length of less than 12 mm, but these tiny crabs almost always died within 18 hr, making any prolonged experimentation impossible. Since no apparent difference was observed in the chromatophore responses of the two crab sizes, the results have been combined.

Crabs were caught either at night or shortly after dawn by chasing them with nets or by digging them out of their burrows. They were placed immediately in individual $8 \times 4 \times 4$ inch plywood boxes with $\frac{1}{2}$ inch mesh wire tops, filled about $\frac{3}{4}$ inch deep with sand. These crabs were returned to the laboratory within an hour after collection, and a petri dish of sea water was put in each box.

Within 8 hr after return to the laboratory the crabs were placed on one of the following regimes.

White background, normal photoperiod
White background, reversed photoperiod
White background, constant darkness
White background, constant illumination
Black background, normal photoperiod
Black background, reversed photoperiod
Black background, constant darkness
Black background, constant illumination

During an experiment the animals' black chromatophores on the proximal, anterior surface of the third walking leg were indexed, using the Hogben and Slome (1931) indexing

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scale at 6-hr intervals with the aid of a Kyowa dissecting microscope (× 45) and a Spencer illuminator. The crabs were kept in their individual wooden boxes with a petri dish of sea water to which fresh sea water was added every 6 hr if needed. They were fed daily, either a small bit of raw meat or bread soaked in milk.

The normal photoperiod was daylight and nighttime with the animals kept outside the laboratory. Two indexing time patterns were used. The 0600, 1200, 1800, 2400 sequence provided three indexing periods in daylight and one in the dark. The 0500, 1100, 1700, 2300 sequence provided two daylight and two dark indexing periods. The animals in constant darkness were kept in a dark closet and were exposed to the light only for the few seconds it required to index their chromatophores every 6 hr. A 15-watt Westinghouse cool white fluorescent light in the closet was used to maintain the animals in constant illumination with the animals kept at a distance of 3.5 ft from the light source. The artificial light and the dark closet were also used to reverse the normal photoperiod by keeping the animals in the dark for 12 hr during the day and turning on the light for 12 hr at night.

White sand from Oahu's beaches provided the white background used in the experiments. Black sand from the island of Hawaii, originating from black lava, was used in the experiments where a black background was needed.

As a final experiment an attempt was made to see if the observed chromatophore responses were the result of visual stimuli. Medium crabs were collected and maintained on a white background. In half of these crabs the eyestalks were completely covered with dark red Revlon nail polish. The eyestalks of the other half of the crabs were covered with clear Revlon polish. The animals were kept outside the laboratory in the normal photoperiod and their chromatophores were indexed every 6 hr at 0600, 1200, 1800, 2400.

RESULTS

The data are presented in Figures 1–6. Time of day is given on the abscissa, and the chromatophore rating scale of Hogben and Slome (1931) is given on the ordinate. Unless otherwise indicated, 10 crabs were used for each experiment. The data have not been treated statistically.

In the normal photoperiod, using the indexing times of 0600, 1200, 1800, and 2400, 20 crabs maintained on a white background displayed a daily rhythmic chromatophore change with maximum pigment dispersion at 0600 and minimum dispersion at 2400 (Fig. 1). The 20 crabs maintained on a black background under the same light conditions displayed a daily rhythm with a peak at 1200 and a low point at 2400 (Fig. 1), though they displayed much less marked response. Crabs on a black background were generally darker at any hour.

Crabs were maintained under the same light conditions with the chromatophores indexed at 0500, 1100, 1700, and 2300 in order to have two indexing periods in the dark (2300 and 0500) and two periods in the light (1100 and 1700). Crabs on white sand maintained a rhythmic change where a peak was reached during the day and a low point at 2300, while crabs on black sand displayed a daily rhythmic change with a similar pattern of peaks and low points (Fig. 2), but with less total variation. These crabs were generally darker at all times.

As shown in Figure 3, crabs maintained in the reversed photoperiod showed a reversal of their daily rhythmic changes. The crabs were in total darkness for 12 hr, including the 1100 and 1700 indexing times, and in constant artificial light for 12 hr, including the 2300 and 0500 indexing times. On both black and white backgrounds the crabs displayed more pigment dispersion in the simulated daytime than in the artificial night. In comparing the reversed (Fig. 3) and normal (Fig. 2) photoperiods, the 0500, 1100, 1700, 2300 normal photoperiod results have been used so that both experiments have two indexing times in the light and two in the dark. Comparison of the chromatophore indices of crabs during the two photoperiods showed that the peaks of the normal photoperiod occurred simultaneously with the low points of the reversed photoperiod and vice versa, indicating that the chromatophore rhythms were reversible. Crabs maintained on white sand displayed this reversal more distinctly than crabs maintained on black sand. Crabs on a black background displayed a greater degree of pigment dispersion at all times.

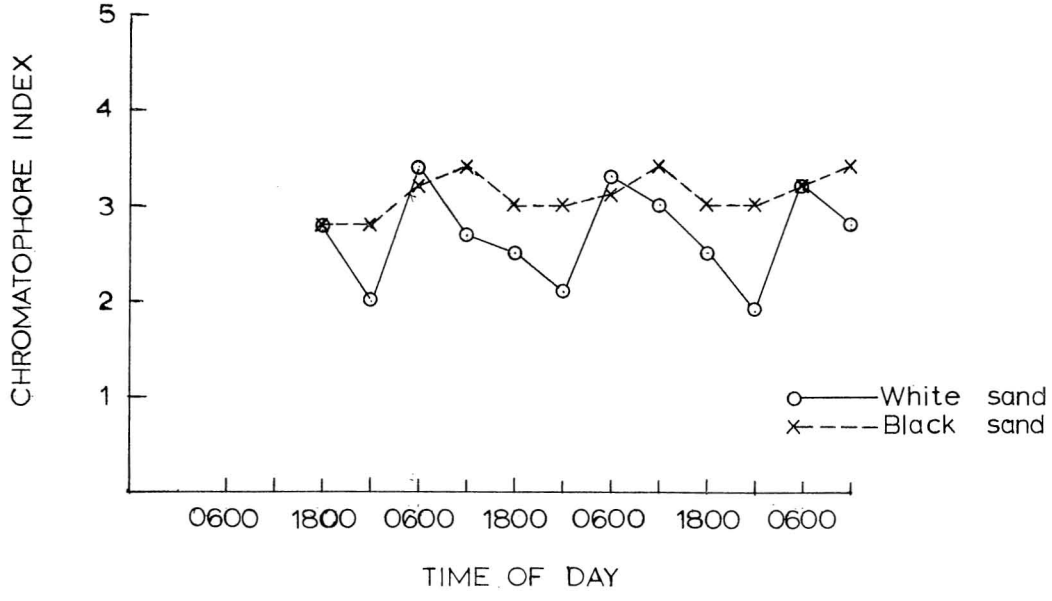

FIG. 1. The average daily indices of the darkly pigmented chromatophores in *Ocypode ceratophthalma.* The crabs were maintained in the normal photoperiod for 3 days on a white or a black background. The chromatophores were indexed every 6 hr.

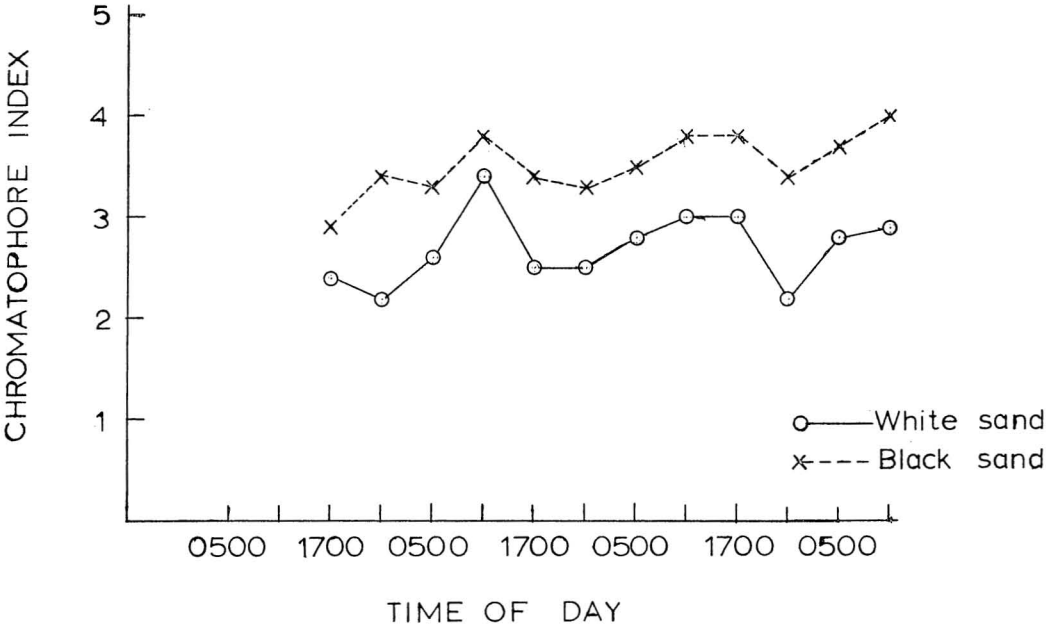

FIG. 2. The average daily indices of the darkly pigmented chromatophores in *Ocypode ceratophathalma.* The crabs were maintained in the normal photoperiod for 3 days on a white or a black background.

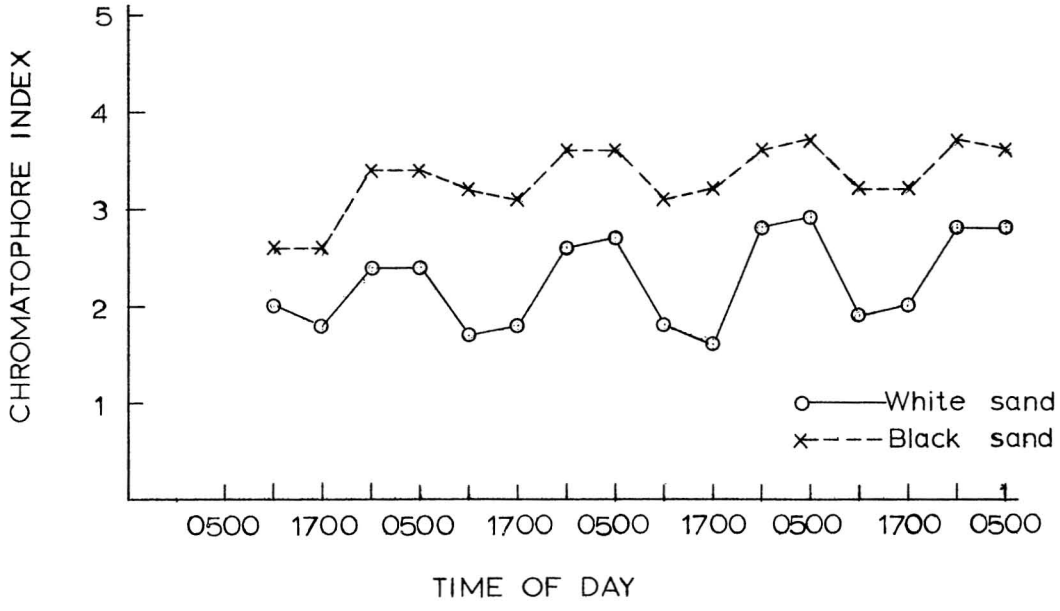

FIG. 3. The average daily indices of the darkly pigmented chromatophores of *Ocypode ceratophthalma* when the crabs were maintained in the artificially reversed photoperiod for 4 days on a black or white background.

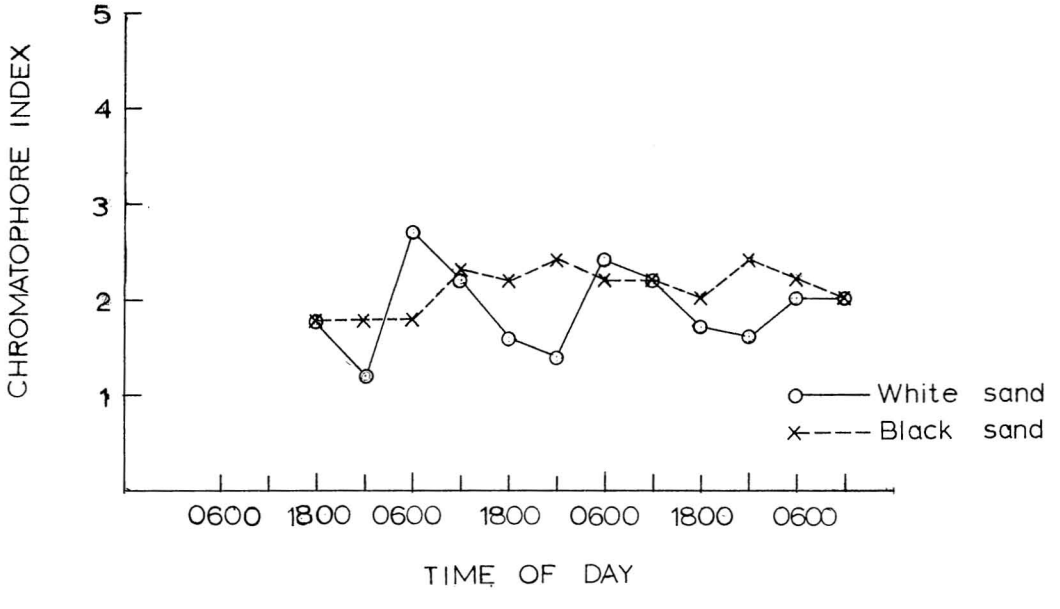

FIG. 4. The average daily indices of the darkly pigmented chromatophores in *Ocypode ceratophthalma* when the crabs were maintained in constant darkness for 3 days on a black or a white background.

Figure 4 shows the results of maintaining crabs in constant darkness. In crabs maintained on white sand a daily chromatophore change rhythm similar to the one displayed during the normal photoperiod was observed, though it was at a lower level on the Hogben and Slome (1931) scale (compare with Fig. 1). The chromatophore rhythm of crabs maintained in constant darkness on black sand became irregular (Fig. 4).

When crabs were maintained on a white background under constant illumination, a small rhythmic chromatophore change was observed the first day, but then it began to decay. In crabs maintained under similar light conditions on a black background the rhythm died out (Fig. 5). As in preceding experiments, crabs on the dark background displayed a consistently greater degree of pigment dispersion.

Figure 6 shows the results of covering the eyestalks of medium-sized crabs with either dark red nail polish or clear nail polish and maintaining them on a white background in the normal photoperiod. When the chromatophores were indexed at night, the two observed indices were very close, but when the chromatophores were indexed in the light, the crabs with the clear nail polish on their eyestalks showed a greater degree of pigment dispersion (Fig. 6), at least during the first two days. Toward the end of the experiment the difference in response between the two experimental groups diminished appreciably. This phenomenon was attributed to chipping of the red nail polish.

DISCUSSION

Other studies have been conducted on the existence of persistent rhythmic physiological chromatophore changes. An endogenous rhythm in the fiddler crab *Uca* has been reported by Brown and Sandeen (1948:370). The persistence of this rhythm in total darkness has been tested (Brown, Fingerman, Sandeen, and Webb, 1953:36), and Webb (1950:336) found that the rhythm could be altered by artificially changing the normal time of night and day.

The results obtained in these experiments suggest that the Hawaiian ghost crab exhibits a daily rhythm of chromatophore changes under normal conditions, with maximum concentration of the dark pigment at night and maximum dispersion of this pigment during the day. In most chromatic decapods the dark pigment in

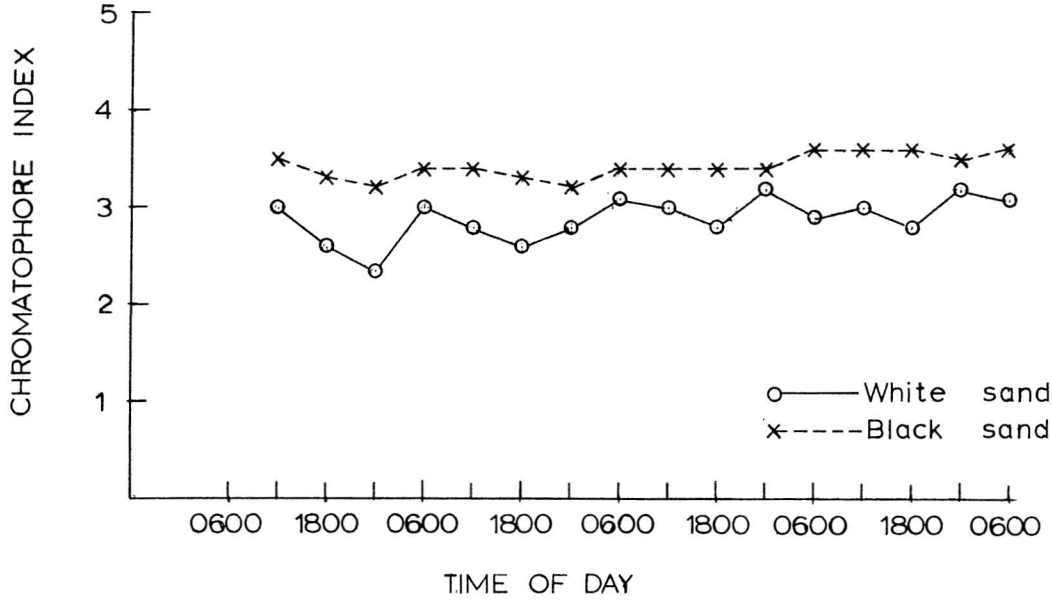

FIG. 5. The average daily indices of the darkly pigmented chromatophores in *Ocypode ceratophthalma* when the crabs were maintained in constant fluorescent illumination for 4 days on a black or a white background.

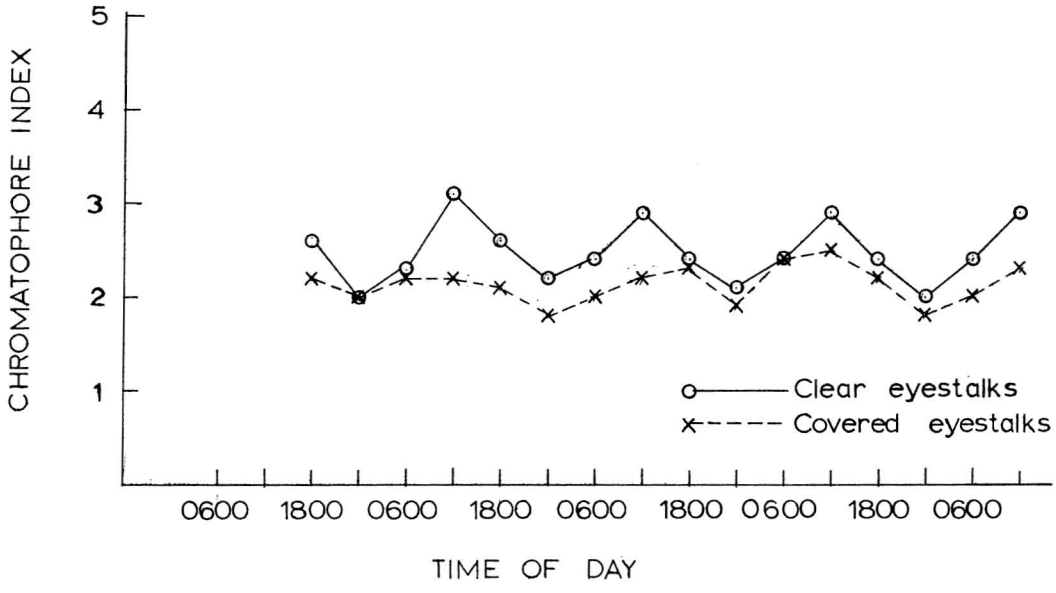

FIG. 6. The average daily indices of the darkly pigmented chromatophores in medium-sized *Ocypode ceratophthalma* when the crabs were maintained in the normal photoperiod on white sand for 4 days with eyestalks covered with clear or red nail polish.

the chromatophores concentrates at night (Parker, 1948:51). On white sand this concentration of pigment at night enabled the crabs to blend with the white background. On black sand there was less blanching at night. This definite change in the normal pattern of chromatophore responses would indicate that the responses are flexible and are influenced not only by the light intensity but by the color of the background as well.

Smith (1938:252) observed in *Ligia* that a sudden change in the color of the background was frequently accompanied by a chromatophore response in which the animal gradually adapted to the new background color. Studies on *Uca* (Brown and Sandeen, 1948:366) showed a greater dispersion of dark pigment in animals on a dark background than on a light background when light intensity was the same.

Constant laboratory conditions were used for the reversed photoperiod experiments. The chromatophore rhythm which was established corresponded to the artificial night and day, with the animals being darkest during the artificial day and lightest during the artificial night, indicating that the rhythmic chromatophore changes can be reversed.

Brown (1961:510) reported that a daily rhythm of chromatophore responses will persist under conditions of constant darkness in many species. In the experiment reported here the results showed that a diurnal rhythm was maintained, although at a lower level on the Hogben and Slome scale when the crabs were maintained on white sand. The normal environment of these crabs is on white sand and in constant darkness, for they are active at night and spend the day underground. The conditions of the experiment conducted in total darkness were, therefore, quite similar to those of their natural surroundings. During the experimental period the crabs were very active, behaving as they normally do at night. Although the chromatophore rhythm was not destroyed under the conditions of constant darkness, it was evident toward the end of the last day that the pattern had started to decay. The crabs maintained in total darkness on black sand, however, showed very little change in pigment concentration with respect to time. Because the black sand is much coarser than the white, one must include the possibility that substrate characteristics other than reflectivity might influence chromatophore responses.

The observed daily chromatophore rhythm did not persist in the experiments in which the crabs were maintained in constant illumination. The light conditions of these experiments were completely foreign to the animals, and the crabs behaved sluggishly. There were no cues from the environment to aid in maintaining the rhythm, and it did not persist. There was no evidence of a persisting diurnal rhythm. The amount of illumination was the same for both groups of crabs, yet those on black sand showed a consistently greater degree of pigment dispersion, showing that there is a response to the color of the background.

In the final experiment an attempt was made to determine whether the observed daily responses were responses to a visual stimulus. Cowles (1905:23–24) reported that after painting the eyestalks of *Ocypode* with lampblack, no further color changes were observed. This observation, however, was not quantitative, for he observed only gross appearance and did not describe the condition of the chromatophores. In the present study the eyestalks of 10 medium crabs were covered with dark red nail polish. While it was uncertain whether this treatment blocked all the light transmission, the response suggested that the red coating effectively reduced visual reception. Normally the ghost crab demonstrates a shadow reflex, depressing its eyestalks when moved from the light into the shade or vice versa. The crabs whose eyestalks were painted with clear polish did demonstrate the reflex; the crabs whose eyestalks were painted red did not. The degree of pigment dispersion in the red-painted crabs was much less. Clear nail polish may have cut out some of the light, which might account for the decrease in amplitude of the daily chromatophore rhythm as compared with crabs with normal eyestalks. The results, however, suggest that the visual reception of light is an important factor in maintaining a daily rhythm of chromatophore changes.

SUMMARY

1. The Hawaiian ghost crab, when maintained on white sand, demonstrates a daily rhythm of chromatophore changes with maximum dispersion of dark pigment during the day and maximum concentration at night. On a black background the same daily rhythm of chromatophore changes is observed, but there is generally less concentration of pigment at all times.

2. In an artificially reversed photoperiod the crabs on both black and white backgrounds display a reversal of the daily rhythm of chromatophore changes.

3. In constant darkness crabs on white sand still display the daily rhythm but at a lower over-all level of pigment dispersion; on black sand the rhythm becomes irregular in constant darkness.

4. Under conditions of constant illumination crabs maintained on both backgrounds show little if any rhythm of chromatophore dispersion and concentration.

5. The observed chromatophore responses are primarily responses to visual stimuli, although in the absence of light evidence is given for an endogenous rhythm and for alteration of rhythm by substrate.

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